Charles University in Prague
Faculty of Pharmacy in Hradec Králové
Department of Pharmaceutical Chemistry and Drug Control

Current trends in the development of antiviral drugs

Hradec Králové, 2013 Olga Ioannidou

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Date: Signature:

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Acknowledgement

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ABSTRACT

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Chemistry and Drug Control

Student: Olga Ioannidou

Consultant: Assoc. Prof. RNDr. Veronika Opletalová, Ph.D.

Title of Thesis: Current trends in the development of antiviral drugs

Since the introduction of the first antiviral drug, a great progress has been made. Every year, many substances are tested for their activity against a wide range of viruses. Especially for serious diseases, like AIDS and hepatitis C, the need for safer and more effective drugs is increased.

In the first part of the thesis older, but still important drugs are described and also their structures are given. Some of them have a wide spectrum of activity, such as ribavirin, which is used for the treatment of both hepatitis C and viral hemorrhagic fever.

In the second part, newly developed drugs and drugs that are under clinical trials are discussed. New drugs have greater efficacy and fewer side effects. Nevertheless, there are some viral diseases that could not be managed with drugs and must be prevented by vaccines. To overcome all difficulties and develop both new drugs and vaccines remains a great challenge for the scientists.

ABSTRAKT

Univerzita Karlova v Praze

Farmaceutická fakulta in Hradec Králové

Katedra farmaceutické chemie a kontroly léčiv

Student: Olga Ioannidou

Konsultant: doc. RNDr. Veronika Opletalová, Ph.D.

Název diplomové práce: Pokroky ve vývoji antivirotik

Od zavedení prvního antivirotika došlo k výraznému pokroku. Každoročně je testováno mnoho sloučenin na antivirovou aktivitu proti širokému spektru virů. Obzvláště proti závažným chorobám jako jsou AIDS a hepatitida C je třeba nalézt účinnější a bezpečnější léčiva.

V první části diplomové práce jsou popsána starší, ale stále důležitá léčiva a jsou uvedeny i jejich struktury. Některá z nich mají široké spektrum účinku, např. ribavirin, který se používá jak pro léčbu hepatitidy C, tak pro léčbu hemoragické horečky.

V druhé části jsou diskutována nově vyvíjená léčiva, z nichž většina je zatím v klinickém zkoušení. Nová léčiva mají vyšší účinnost a slabší vedlejší účinky. Avšak stále existují virové infekce, které nelze zvládnout podíváním léčiv a musí se jim předcházet vakcinací. Překonat všechny těžkosti a vyvinout nová léčiva a vakcíny je pro vědce stále velkou výzvou.

ABBREVIATIONS

DNA**:** deoxyribonucleic acid

RNA: ribonucleic acid

m-RNA: messenger RNA

sRNA: bacterial small RNA

mi-RNA: micro-RNA

ssDNA: single-stranded DNA

HIV: Human Immunodeficiency Virus

HSV-1, HSV-2: Herpes Simplex Virus type 1 and 2

HCMV: Human Cytomegalovirus

VZV: Varicella-zoster Virus

AIDS: Acquired Immunodeficiency Syndrome

CD4: *cluster of differentiation 4*

HAV: Hepatitis A Virus

HBV: Hepatitis B Virus

HCV: Hepatitis C Virus

Ig: Immunoglobulin

INF-α: Interferon alpha

RVF: Rift Valley Virus

NK: Natural killer cells

TLR: Toll-like receptor

1. INTRODUCTION

Viruses are non-cellular forms of life composed of proteins and a nucleic acid, which is enclosed in a protective capsid. The first virus that was described was tobacco mosaic virus in 1892 by Dmitri Ivanovsky. Since then, about 5.000 viruses have been discovered and also many ways of the treatment of viral infection.[1]

Viruses have many differences that characterize them from microorganisms: they contain only one type of nucleic acid (DNA or RNA), but never both of them simultaneously and also, they do not have ribosomes or other cellular organelles. This is the reason why all viruses demand a host cell in order to multiply and outside of it they are inactive.[2]

There are several types of viruses depending on:

- The type of nucleic acid that they contain
  - Viruses with double-stranded DNA, *e.g.* **adenovirus, herpes virus, poxvirus, vaccinia virus**
  - Viruses with single-stranded DNA, *e.g.* some bacteriophages
  - Viruses with double-stranded RNA, *e.g.* **retrovirus**
  - Viruses with single-stranded RNA, *e.g.* **poliovirus, influenza virus, HIV, RNA oncogenic viruses**
- The type of cells that they infect
  - Animal virus
  - Plant virus
  - Bacterial virus or bacteriophages [1,2]

Each virus affects only some cells (*e.g.* the HIV virus affects $CD4^+$ T cells and macrophages). The multiplication cycle of viruses consists of four main parts. First is the entrance of the virus into the host-cell. Immediately after, the expression of viruse's genes, the multiplication of the nucleic acid and the synthesis of proteins follows. New virions are formed and released from the host-cell by cell lysis.[3]

2. AIM OF THE WORK

For hundreds of years, people were trying to find safe ways of the treatment of viral infections. But this was found to be very difficult due to the ability of viruses to use the host-cell in order to replicate, so the drugs may cause harm also to host cells.[4]

Nevertheless, today some methods are used in order to treat or minimize the symptoms caused by the viral infection. According to the mechanism of action old and newly produced drugs can be classified in three basic classes:

- Inhibition of the entrance to the host cell
- Inhibition of specific viral enzymes, such as reverse transcriptase, that is used by viruses in order to generate DNA from RNA, and RNA polymerase, that does not exist in the host cell.
- Inhibition of viral proteins production, such as in the case of HIV virus.[5]

In that diploma thesis, I will concentrate on newly developed drugs for the most important diseases caused by viruses, and the older drugs that have been used until today will be mentioned only briefly. The viruses that cause the most common diseases and that will be explicated later in detail are:

- **Herpes virus:** there are five types of this virus that are widely spread among humans. ***Herpes simplex*** (HSV-1), ***genital herpes*** (HSV-2)***, Epstein-Barr virus***, ***Varicella-zoster virus*** and ***Cytomegalovirus***
- **Hepatitis virus:** causes 3 types of hepatitis, Hepatitis A, B and C
- **HIV virus:** causes AIDS (acquired immunodeficiency syndrome)
- **HPV virus:** causes warts (non-cancer skin growths) and in some cases, cervix or vagina cancer
- **Influenza virus:** causes flu, the most common worldwide viral infection.[6]

3. OVERWIEW OF OLDER ANTIVIRAL DRUGS

Since 1960's many antiviral drugs have been discovered and gave us the opportunity to deal with many viral infections that we could not before. Most of them are efficiently used nowadays, while new substances are tested in order to produce new, safer and more efficient drugs.[7]

3.1. Herpetic infections

Herpetic infections are very common among human population and sometimes can lead to serious diseases, such as cancer. The cycle of herpes virus is characterized by activation and remission periods.[8]

In the active period, virus-containing blisters occur and this situation can last several days, or sometimes, up to three weeks. In that period of time the virus becomes contagious and other people can be easily contaminated by direct skin contact or body fluids. On the other hand, in the remission period the virus stays in the sensory nerves of the central nervous system (CNS) without causing symptoms of infection (asymptomatic period).[9]

Classification of Herpes viruses:

- **Herpes simplex type 1 (HSV-1) and type 2 (HSV-2)**

  These types of herpes virus cause most commonly oral and genital herpes, presenting with blisters on the lips and vesicles on the surface of genitals. They are transmitted either by direct contact or by sexual intercourse.

- **Epstein-Barr virus**

  It is responsible for transmission of *infectious mononucleosis* accompanied by flu-like symptoms, such as fever, sore throat and fatigue. It is transmitted by saliva.

- **Varicella-Zoster virus**

  It causes chickenpox in children and herpes zoster in adults. The main symptoms are red rashes throughout the body, fever and malaise. The disease in the adulthood usually causes more serious symptoms.

- **Human Cytomegalovirus**

  It causes disease, which is associated with salivary glands. It is most commonly

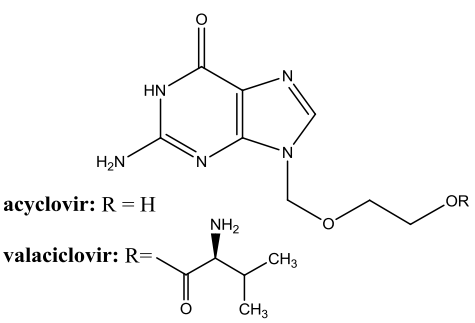
reported in immunocompromised people affected with HIV virus. It appears rarely in healthy people.[10]

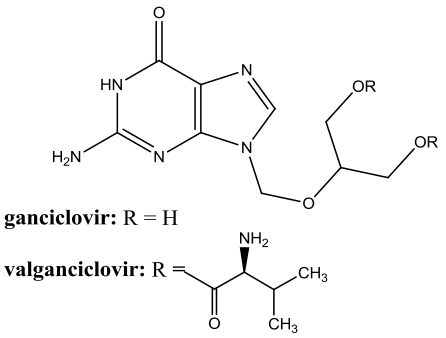
3.1.1. Drugs used for all types of herpetic infections

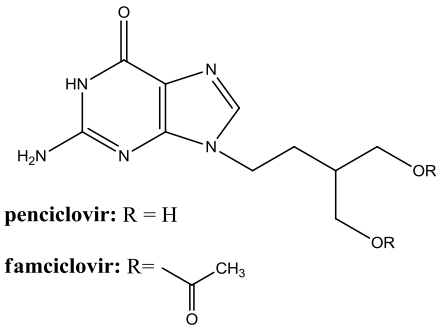
3.1.1.1. Herpes simplex (HSV-1) and Genital herpes (HSV-2)

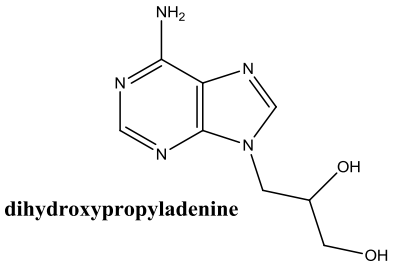
3.1.1.1.1 Acyclic Nucleosides

The identification of **acyclovir** as a selective inhibitor of herpes simplex virus (HSV) replication was an important breakthrough in the development of antiviral drugs. Its selectivity is based on a specific recognition by the HSV-encoded thymidine kinase. Gradually, the derivatives of acyclovir with improved properties have been developed. [11]. **Ganciclovir** is effective also against CMV infections.[12] **Valaciclovir** and **valganciclovir** are prodrugs that are *in vivo* converted to the parent drug. **Penciclovir** is the active metabolite of the oral product **famciclovir.**[13]





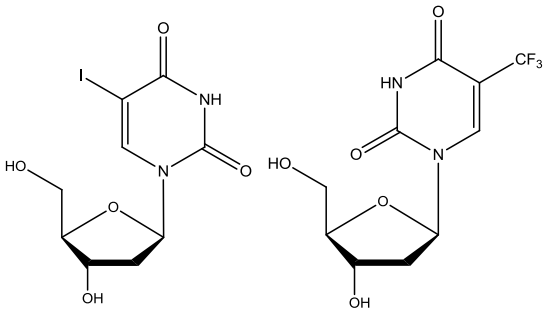




Acyclovir is converted to acyclovir triphosphate by enzyme thymidine kinase and then it is incorporated into the viral DNA.[14]

3.1.1.1.2 Nucleoside analogues

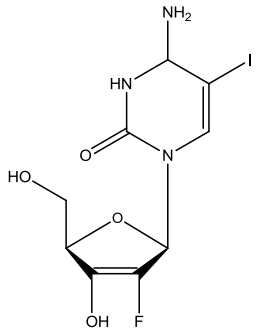
Idoxuridine is a nucleoside analogue used only topically for treatment of herpes simplex keratitis. **Trifluridine** is used topically in the eye for treatment of herpetic infections.[13,15]



idoxuridine **trifluridine**

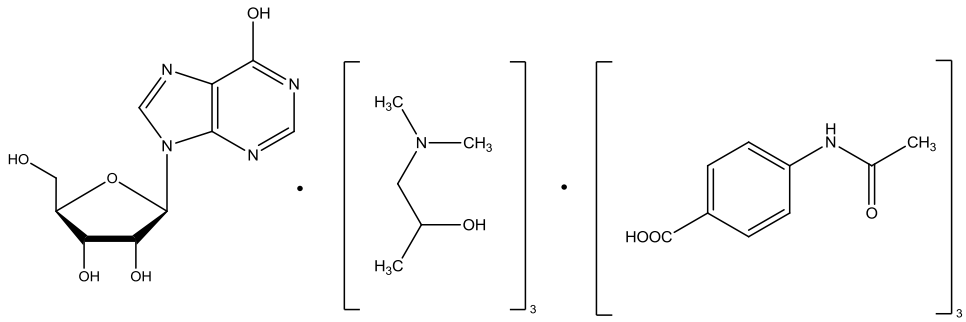
3.1.1.2. Epstein-Barr virus

Epstein-Barr virus causes infectious mononucleosis and oral hairy leukoplakia. One of the prospective drugs against this virus was a DNA polymerase inhibitor **fiacitabine.** However, has been not widely used in clinical practice. [16, 17]



fiacitabine

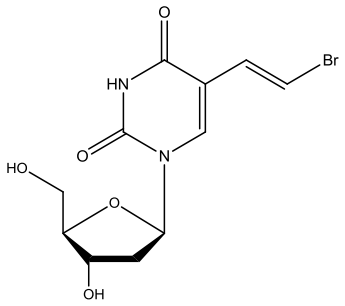
Inosine pranobex is a complex of inosine with dimepranol acedoben – (±)-1-(dimethylamino)propan-2-ol *p*-acetamidobenzoate). It has been used in the treatment of subacute sclerosing panencephalitis and herpes simplex infections.[18].



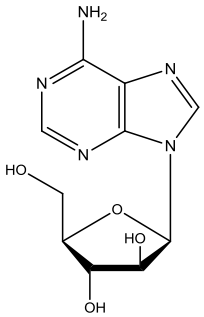
inosine pranobex

3.1.1.3. Varicella-Zoster virus

Brivudine is a nucleoside thymidine analogue. Mechanism of action includes incorporation into the viral DNA and inhibition of viral replication by blocking DNA polymerases.[19] **Vidarabine** is a nucleoside analogue used systematically against varicella-zoster and herpes simplex infections. Mechanism of action is similar with acyclovir.[13]



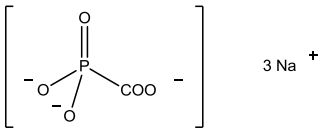
brivudine



vidarabine

3.1.1.4. Human Cytomegalovirus

Foscarnet is a non-nucleoside pyrophosphate analogue active against herpes viruses. The pyrophosphate binds to the viral DNA polymerase. It is used as the trisodium salt mainly for the treatment of CMV retinitis in AIDS patients and for aciclovir-resistant herpes simplex virus infections in immunocompromised patients.[13]



foscarnet

Fomivirsen is a synthetic, phosphorothioate antisense oligonucleotide designed to inhibit gene expression in human cytomegalovirus (CMV). It is consisted of 21 nucleotides in a sequence complementary to the specific portion of CMV messenger RNA which encodes regulatory proteins. It has been used as the sodium salt for the local treatment of CMV retinitis in patients with AIDS.[20]

5′-d-[G*C*G*T*T*T*G*C*T*C*T*T*C*T*T*C*T*T*G*C*G]-3′
sodium salt

* = racemic phosphorothioate

fomivirsen

3.2. HIV infections

HIV (Human Immunodeficiency Virus) tends to be a modern plague. It infects T helper cells ($CD4^+$ T cells) and macrophages, important immune system cells. This leads to a failure in the patient's immune system, which cannot deal even with the simplest infections. People infected with HIV virus usually suffer from *Pneumocystis pneumonia* and CMV keratitis.[21]

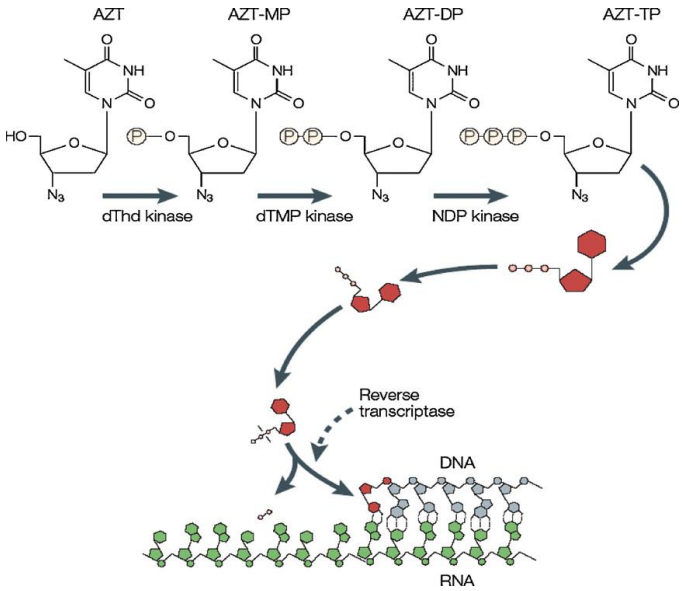
There are recognized two types of HIV virus, HIV-1, which causes more serious effects and HIV-2 with low transmission ability. The virus's genome is single-stranded RNA and that is the reason that the enzyme reverse transcriptase, which converts RNA into DNA, is needed. The HIV has high mutation ability, so vaccination is ineffective at this situation. The virus is mainly transmitted via blood, breast milk and semen.[22]

3.2.1. Drugs used for HIV infections

3.2.1.1. Nucleoside inhibitors of reverse transcriptase

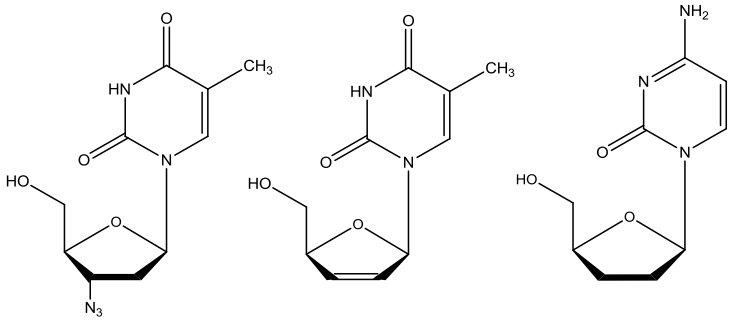
Didanosine is a dideoxynucleoside, reverse transcriptase inhibitor and it is combined with other antiretroviral agents, such as **stavudine**, for the treatment of HIV patients. Its mechanism of action is an early termination of the viral genetic material synthesis. **Zidovudine** is used in post-exposure prophylaxis together with **lamivudine.**

Mechanism of action of zidovudine is illustrated in the following picture.

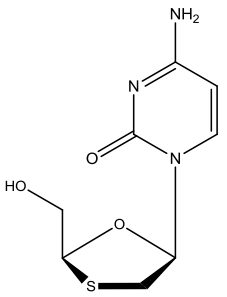


Mechanism of action of zidovudine (AZT). Following phosphorylation to its triphosphate form (AZT-TP), AZT acts as a competitive inhibitor/alternative substrate with respect to dTTP in the reverse transcriptase reaction [23]

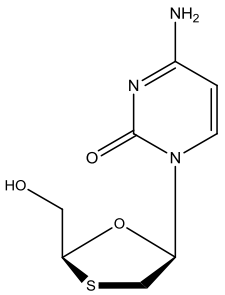
Zalcitabine is a pyrimidine analogue and is less potent than the other drugs of this group. Similarly, **emtricitabine** (a pyrimidine antimetabolite) **abacavir** (a guanosine analogue) are always used in combination with other antiretroviral agents.[13,22-24]



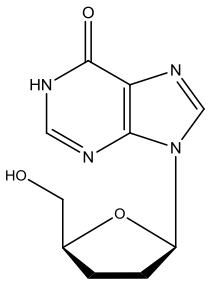
zidovudine **stavudine** **zalcitabine**



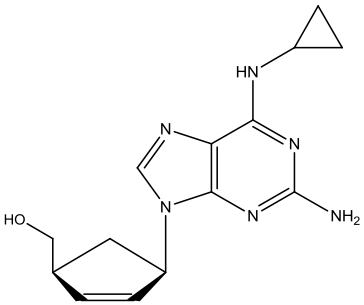
lamivudine



emtricitabine



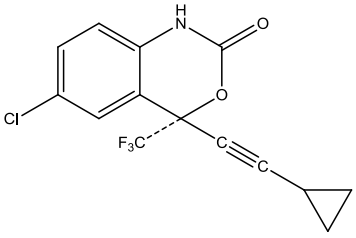
didanosine



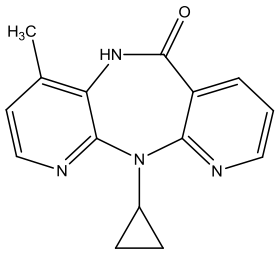
abacavir

3.2.1.2. Non-nucleoside inhibitors of reverse transcriptase

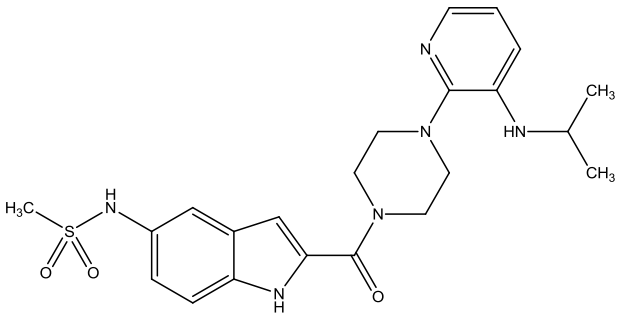
These compound inhibit reverse transcriptase in a non-competitive manner.[25]



efavirenz



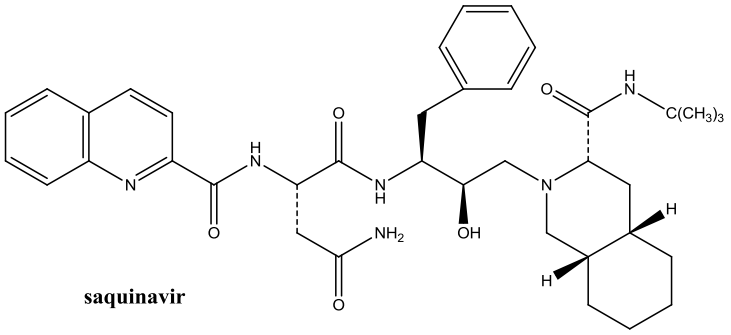
nevirapine



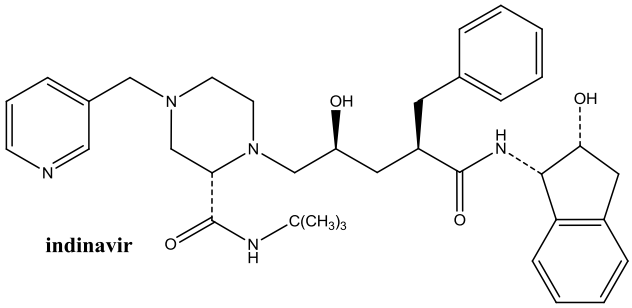
delavirdine methansulfonate

3.2.1.3. HIV protease inhibitors

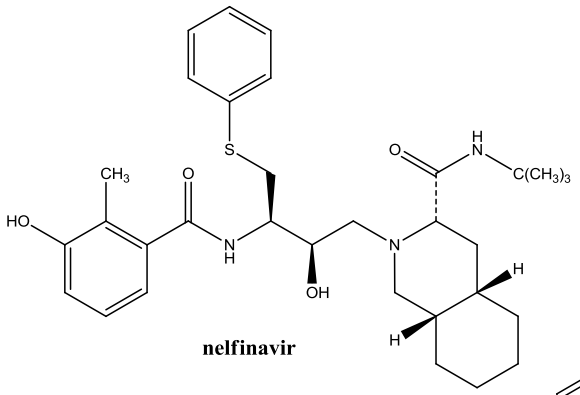
This class of drugs inhibits the proteases, enzymes that cleave proteins into smaller fragments.[23]



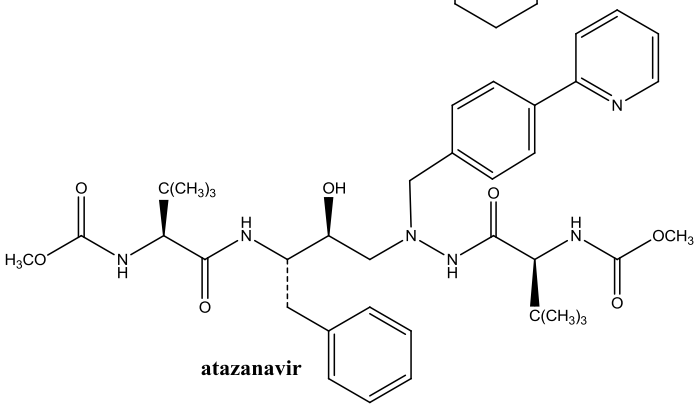
saquinavir



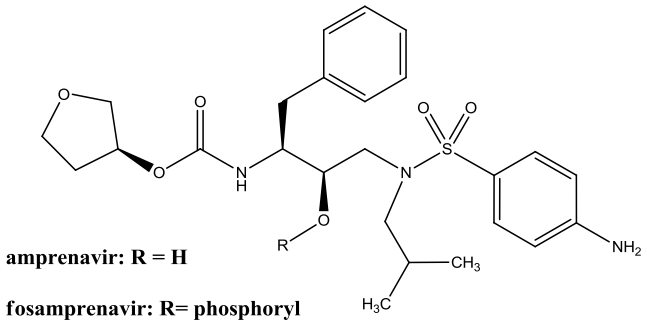
indinavir



nelfinavir



atazanavir

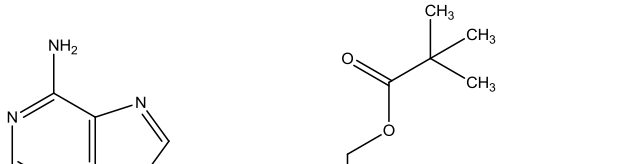


amprenavir: R = H

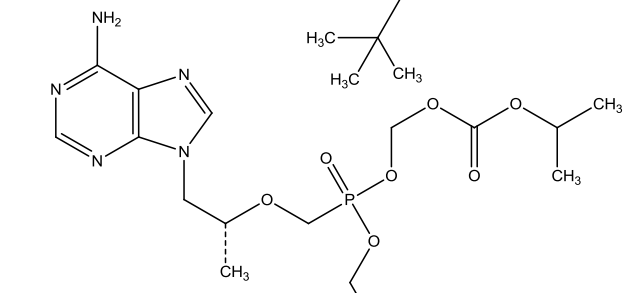
fosamprenavir: R= phosphoryl

3.2.1.4. Phosphonates of acyclic nucleosides

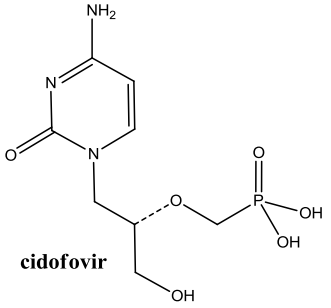
This is a class of compound that inhibit of reverse transcriptase as well as DNA polymerase.[26]



adefovir-dipivoxyl



tenofovir diisoproxil



cidofovir

3.2.1.5. Other drugs

Enfuvirtide is a synthetic, 36 amino acid peptide, corresponding to a region of gp41, the transmembrane subunit of the HIV-1 envelope protein. It is a synthetic fusion inhibitor and not active against HIV-2 type.[27]

3.3. Influenza infections

Influenza or "flu" is a disease caused by influenza viruses. The symptoms of this infectious disease are fever, chills, fatigue, weakness and headache. It can be very easily spread by nasal secretions or direct skin contact. This infection could be very serious and causes more than 300.000 deaths per year.[28]

Three types of influenza virus exist:

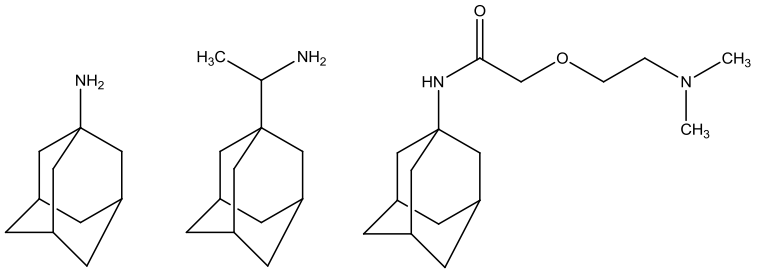
- Influenza virus A, the most dangerous type. It is divided into several serotypes, including H1N1 and H5N1. H1N1 type is well-known for causing Swine flu epidemia in 2009 and H5N1 for causing Bird flu in 2004
- Influenza virus B
- Influenza virus C [29]

This virus has high mutation capacity, so vaccines must be renewed every few years. Vaccination is proposed in high-risk groups, such as children, asthmatic and diabetic patients and elderly.[30]

3.3.1. Drugs used for influenza infections

3.3.1.1. M2 ion channel inhibitors

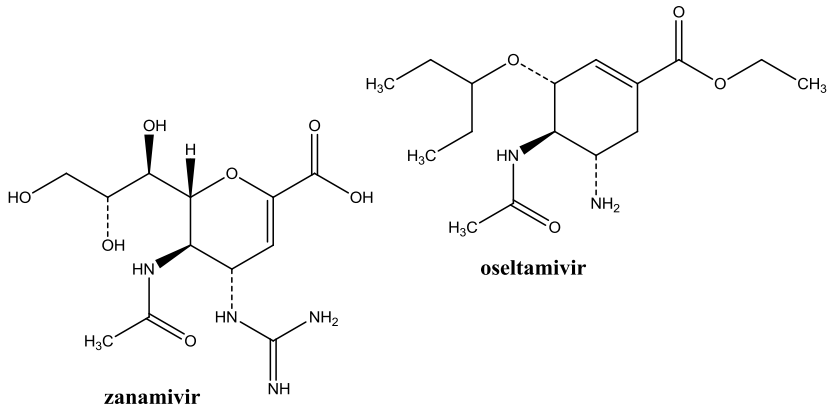
Adamantane derivatives inhibit M_2 protein synthesis. M_2 protein is found in the envelope of influenza virus and plays a very important role in its reproductive cycle. [31]



amantadine **rimantadine** **tromantadine**

3.3.1.2. Neuraminidase inhibitors

Neuraminidase is an enzyme used by the influenza virus in order to be release from the infected cell. Inhibition of the enzyme prevents liberation of new viruses into the blood.[32]



3.4. Hepatitis

Hepatitis is a condition which causes inflammation of the liver. This disease can result either from viral infections or alcohol abuse, medicaments and immune system failure. Hepatitis can be asymptomatic, but in several cases the patient present nausea, vomiting, jaundice, malaise or anorexia. It could potentially lead to liver cirrhosis. Hepatitis is classified into three basic types:[33]

- **Hepatitis A**: Hepatitis A is transmitted via Hepatitis A virus (HAV). It causes acute form of the disease (unlike type C, which causes chronic hepatitis) and can be spread by infected blood, stool or skin contact. Vaccination has already been developed for prevention of infection.
- **Hepatitis B**: This type is transmitted via Hepatitis B virus (HBV) and can be acute or chronic. Vaccination is used for prevention of this disease.
- **Hepatitis C**: It is caused be Hepatitis C virus (HBC) and it is the most harmful strain of hepatic viruses. Most of the patients will need a liver transplantation after the infection with HBC. Vaccine for prevention of Hepatitis C is currently not available.[34]

3.4.1. Drugs used for hepatic infections

3.4.1.1. Hepatitis A

It is has been found that Hepatitis A Immune Globulin (Ig) can be used in treatment or prevent the serious symptoms of Hepatitis A if the patient is recently exposed to the virus. It can be used parallel with the vaccine against hepatitis A virus, which is approved to be very effective.[35]

3.4.1.2. Hepatitis B

There is no specific treatment for acute Hepatitis B. Some antiviral drugs, such as telbivudine, clevudine, laminudine, adefovir and entecavir are used combined with interferon alpha 2α (INF-α) and pegylated interferon alpha 2α. Details about interferons and their mechanism of action will be explained in another chapter.[35]

3.4.1.3. Hepatitis C

Drugs concerning hepatitis C are relatively new and many are under development. They will be discussed in detail later in another chapter.

3.5. Viral hemorrhagic fever

Viral hemorrhagic fever is a medical term for a group of diseases that caused by 4 types of RNA viruses: arenaviruses, filoviruses and bunyaviruses and flaviviruses. It is endemic in Central and West Africa, but can be also found in some regions of South America.[36] The symptoms can be ranged from mild to even severe. In addition to fever, patients also present hemorrhage, nausea and sometimes jaundice. This disease can lead to shock or ever death in serious cases. It is transmitted via direct contact of infected body fluids, such as urine, feces or saliva. Until nowadays, effective vaccine against viral hemorrhagic fever has not be discovered.[37]

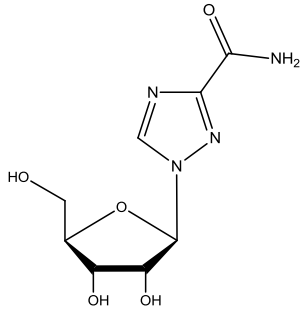
There are several diseases that are cause by these types of viruses.

- Lassa fever, transmitted by Lassa virus of genus Arenavirus. It is spread mainly by rats in West Africa.
- Rift Valley fever, which is transmitted by RVF virus of genus Bunyavavirus.
- Ebola virus disease, transmitted by Ebola virus of genus Filovirus
- Yellow fever, caused by yellow-fever virus of genus Flavivirus. It is mainly

transmitted through the bite of *Aedes aegypti* mosquito.[38]

3.5.1. Drugs used for viral hemorrhagic fever

Ribavirin is the most known drug for the treatment of many kinds of viral hemorrhagic fever. It can also be used for the treatment of Hepatitis C (combined with pegylated interferons) and influenza. [38,39]



ribavirin

3.6. Interferons

Interferons are naturally occurring proteins (glycoproteins) released by virus infected cells and act on neighbouring cells to render them less susceptible to a wide variety of DNA and RNA viruses. Their action is also the activation of important immune cells, such as macrophages and natural killer cells (NK).They are relatively specific for a species and they differ from cell to cell.[40]

There are three classes of interferons:

- Interferon α (alpha) is released from leucocytes and used in the treatment of viral infections (like hepatitis B and C) and some cancer types.
- Interferon β (beta) is released from fibroblasts and used for the treatment of multiple sclerosis.
- Interferon γ (gamma) is released from lymphocytes and used for chronic granulomatous disease.[41]

Mechanism of action of interferons is complex and differs among the various cell types and viruses. Interferons bind to specific cell-surface receptors and inhibit viral penetration, synthesis or methylation of mRNA, translation of viral proteins, or viral assembly and release. Most prominent effect is to inhibit viral protein synthesis, but in some viruses, there is no inhibition of viral RNA or protein synthesis. Following binding to cell surface, interferons may induce synthesis of new cellular RNAs and

proteins which mediate the antiviral effect.

In other words, interferons:

- can enhance cytotoxic activities of lymphocytes
- inhibit cell proliferation
- inhibit expression of cell surface antigens
- inhibit phagocytic and tumoricidal activities of macrophages

Interferons are used in several kinds of viral diseases and more common in the treatment of chronic hepatitis B and C. It has been found that pegylated interferon α is more effective when is combined with ribavirin, rather than when administered alone. Nowadays, it is proposed as a first-line treatment. However, it is not effective against the acute form of hepatitis B and C. [42]

3.6.1. Interferon inducers

Interferon inducers are agents that stimulate the production of endogenous interferons. Cytokines such as interleukin-1, interleukin-2, CSF (colony stimulating factor) and TNF (tumor necrosis factor) can stimulate the interferon production. Although interferon inducers seem to be a good approach in the treatment of viral infection, generally have not be found to be safe and effective and that is the reason that they are not used in clinical practice.[43]

4. PRESENTATION OF NEW ANTIVIRALS

Developing new antiviral drugs is difficult especially because the new agents must subject in certain regulations. They must be effective to one or more kinds of diseases, safe enough in order to limit undesirable effects and also to pass qualitative analysis.

Discovery of absolutely new active substances is rare. Generally, a prototype structure (lead) is discovered. This substance has many undesirable characteristics, such as low absorption capacity or many side effects. That is the reason that new drug structures are developed by the improvement of already existing pharmacological substances, *e.g.* by adding a group into the basic skeleton of the compound in order to improve lipophilicity, absorption and duration of the action and also to avoid some serious adverse effects.

In order for a drug to be approved for registering it must pass preclinical and clinical trials. Preclinical trials include animal testing of the active substance of the drug in order to obtain information about pharmacokinetic and pharmacodynamic characteristics and its physicochemical properties. On the other hand, clinical evaluation is composed of four stages (phases):

- **Phase 1:** it is performed on healthy volunteers in order to obtain information about the safety and tolerance of the drugs
- **Phase 2:** it is performed on a small number of patients, in order to find out the optimal dosage of the drug
- **Phase 3:** a larger number of patients is used. At the end of this phase the drug producer submits the new drug application (NDA), which shown the safety and efficacy of the drug
- **Phase 4:** research on the drug after its approval. The medicine treated patients record all the possible side effects.[44-49]

4.1. New drugs for herpetic infections

Although already approved drugs for the treatment of herpes simplex and are highly effective, there is a need for newly developed substances for the treatment of resistant viruses and for the prevention of viral reactivation.[50]

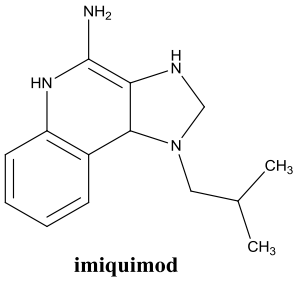
4.1.1. Herpes simplex

4.1.1.1. TLR agonists

Toll-like receptors are proteins that are found in immune system cells, like macrophages and dendritic cells, and help them to recognize special microbe-derived molecules. As a result, they enhance the immunity of the organism against infections. There are several types of toll-like receptors located mainly in the cell surface (TLR-1–TLR-13).

Several experiments have shown that TLR (toll-like receptor) agonists lead to the stimulation of the immune response. It was shown that mice treated with polycytidylic acid, which is a TLR-3 agonist, survived for sufficiently longer time than with TLR-4 or TLR-5 agonists.[51]

Imiquimod is a topical TLR7/8 agonist that has been given as a treatment of rare skin cancers, such as malignant melanomas, but has also shown positive results in the reduction of the warts caused by HSV-2 virus when applied twice a week. It is a relatively old drug which nowadays undergoes further clinical studies in order to be approved for the treatment of herpetic infections.[52]



imiquimod

4.1.1.2. Helicase/primase inhibitors

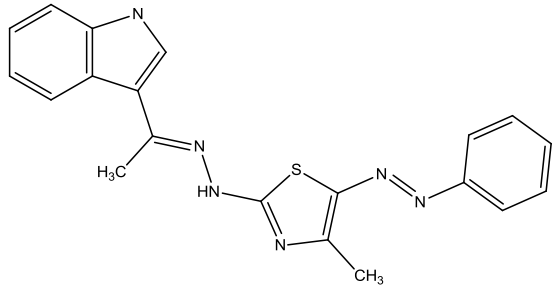
Helicase is an enzyme with the function to unroll the genetic material (DNA or RNA) and plays an important role in replication, transcription and translation. Primase is also an enzyme that converts sRNA to ssDNA and has primary importance to DNA replication.

This category of drugs is under experimental methods with not known clinical experience. The candidate drug BAY 57-1293 was found to be more effective than acyclovir in the treatment of genital and ocular herpes infection in laboratory animals. [53]

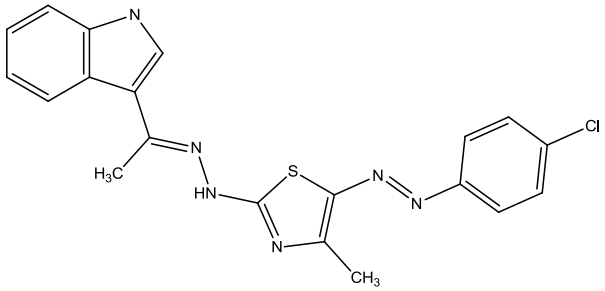
4.1.1.3. Indole-based heterocycles

It is known that indole has many pharmacological properties including anti-inflammatory, antidepressant, antiproliferative and antiherpetic by inhibiting HSV-1intergrase. The goal now is to synthesize new indole derivatives with improved antiviral activity.

Several experiments in animals have shown that some indole-containing compounds *e.g.* 2-{2-[1-(1*H*-indol-3-yl)ethylidene]hydrazinyl}-4-methyl-5-(phenyldiazeny)thiazole and 2-{2-[1-(1*H*-indol-3-yl)ethylidene]hydrazinyl}-5-[(4-chlorophenyl)diazenyl]-4-methylthiazole have shown positive results in reducing of the plaques of Herpes simplex type 1 (HSV-1).[54]



2-{2-[1-(1H-indol-3-yl)ethylidene]hydrazinyl}-4-methyl-5-(phenyldiazeny)thiazole



2-{2-[1-(1*H*-indol-3-yl)ethylidene]hydrazinyl}-5-[(4-chlorophenyl)diazenyl]-4-methylthiazole

4.1.1.4. Anti-heparan sulfate peptides

The molecule of heparin sulfate (HS) and one modification form of it, 3-*O*-sulfated heparan sulfate (3-OS HS) work as attachment site for HSV-1 virus. 3-*O*-sulfated heparan sulfate is produced enzymatically from HS by 3-*O*-sulfortransferases and works as a mediator of the penetration of HSV-1 virus into the cells. Two different anti-HS peptides were tested against HSV-1, G1 peptide (LRSRTKIIRIRH) and G2 peptide (MPRRRRIRRRQK). Both of them successfully blocked the entry of the virus into the

cell and with further future experiments it will be determined if anti-HS peptides could be used as a first-line treatment against HSV-1.[55]

4.1.2. Varicella zoster

4.1.2.1. Salivary micro-RNAs

MicroRNAs (miRNAs) are non-coding RNA type that bind and suppress complementary mRNA targets. In our case, the target is m-RNA of VZV. These mi-RNAs are found in the salivary microvesicles of humans and could be used as effective antiviral agents against ophthalmic herpes zoster caused by Varicella-zoster virus (VZV).

Microvesicles contain about 20 mi-RNAs, which have the ability to inhibit the VZV's mRNAs and, as a result, the inhibition of their translation and replication. As a mechanism of action, it is suggested that human salivary mi-RNAs are bound to plasma proteins and enter the corneal cells of the eye via endocytosis.[56]

4.1.3. Human Cytomegalovirus

4.1.3.1. Peptides targeting glycoprotein b (gb)

Glycoprotein b is a glycoprotein found on the outer layer of the envelope of herpes viruses. It works as a "fusion protein" and it is required for the entry of the virus into the cells. Due to the increased need for better anti-HCMV drugs with decreased side effects and cytotoxicity, therapeutic peptides which target glycoprotein g5 of human cytomegalovirus were developed as potent antivirals.

It was found that gb contains numerous regions, which have the ability to interact with lipid bilayers and hydrophobic surfaces of the proteins. Several peptides at many concentrations were incubated with human fibroblastic cells and afterwards the cells were infected with HCMV. The results were positive and exhibit inhibition of HCMV infection with a range between 60% – 97%, depending on the type of the used peptide. As a result, gb inhibition peptides could be a basis for new therapeutics for the prevention of Human cytomegalovirus infection.[57]

4.1.4. Epstein-Barr virus

Concerning the treatment of Epstein-Barr virus, there are not newly developed drugs for this strain of herpes virus. Several agents that treat other herpetic infection could be

used, including acyclovir, ganciclovir and foscarnet. A vaccine is under development[16].

4.2. New drugs for HIV infections

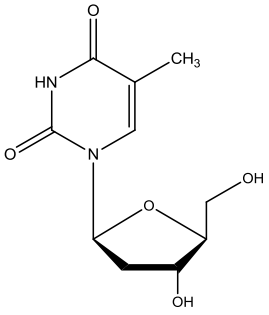
Treatment of Human Immunodeficiency virus is difficult and complex, due to the ability of the HIV virus to mutate. Modern therapeutic pattern is HAART (Highly Active Antiretroviral Therapy), in which combinations of three or even more antiviral agents are used (usually combination of non-nucleoside reverse transcriptase inhibitors, HIV protease inhibitors and nucleoside reverse transcriptase inhibitors). HAART has succeeded in the decrease of the amount of virus present in blood and in the increase in life expectancy.[22,58]

4.2.1. Nucleoside reverse transcriptase inhibitors

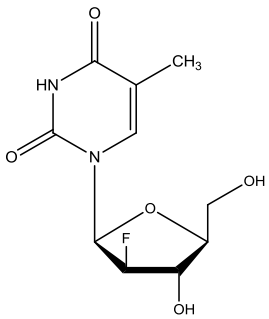
Telbivudine, **clevudine** and **entecavir** belong to nucleoside reverse transcriptase inhibitors and are registered as antiviral drug against chronic hepatitis B. They are also active in a high range of retroviruses, such as HIV. A recent research demonstrated that could be also used in HIV-1/HBV co-infection. In a recent treatment of a patient positive in HIV and hepatitis B, both treated with adefovir dipivoxyl and telbivudine, it was found that when administration of telbivudine discontinued the viral load of HIV was significantly increased. When it was administered again after a two-week interval, the viral load was decreased. This situation could be a major step for future approval of telbivudine as an anti-HIV-1 agent.[59]

Clevudine is a unique antiviral nucleotide analogue because its antiviral activity persists after discontinuation of therapy, as demonstrated by in vitro and in vivo trials was recently approved for HBV infected patients infected with HIV. Unfortunately, it can induce myopathy.[60,61]

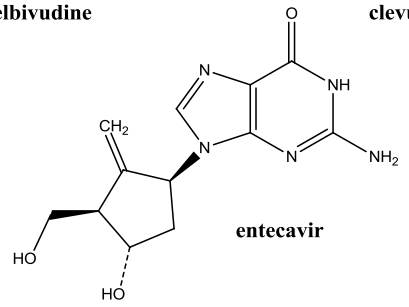
Similarly, **entecavir** is used for the treatment of chronic hepatitis B and it is also approved for chronic hepatitis C and HIV co-infection. In entecavir, the sugar moiety is replaced with an oxygen non-containing five membered ring.[62]



telbivudine



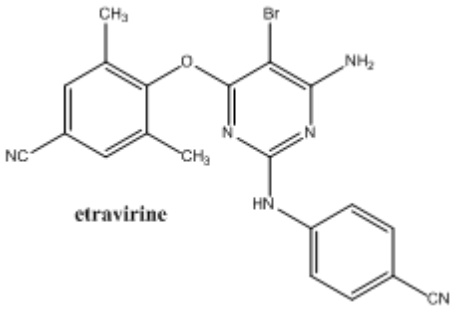
clevudine



entecavir

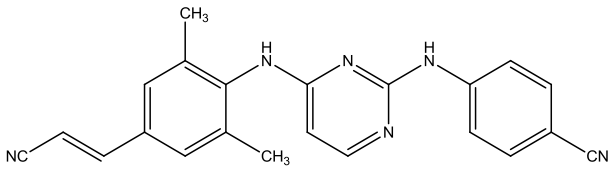
4.2.2. Non-nucleoside reverse transcriptase inhibitors

Etravirine belongs to the second generation of non-nucleoside reverse transcriptase inhibitors and it is indicated for the treatment of HIV-1. It is a diarylpyrimidine molecule that inhibits the enzyme reverse transcriptase in a non-competitive manner and leads to the increased amount of $CD4^+$ T cells. It's advantage is that etravirine is active even against specific viral mutation, in which efavirenz and nevirapine are not active.[63]



etravirine

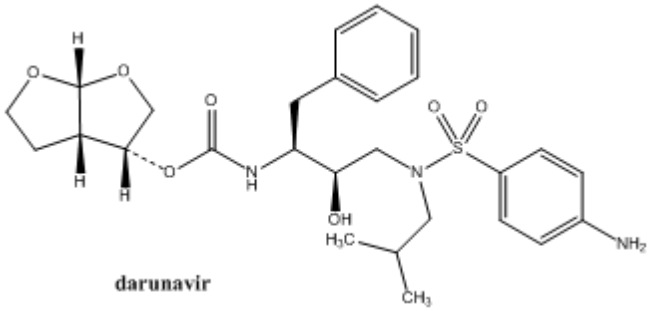
Rilpiverine is a second generation of nucleoside reverse transcriptase inhibitors, which exhibits lower side-effects, compared to other drugs of the same category (but can lead to hepatotoxicity). It is a diarylpyrimidine and it is used as a combination with other antiviral agents like emtricitabine and tenofovir, in order to increase the therapeutic potency against HIV.[64]



rilpiverine

4.2.3. HIV protease inhibitors

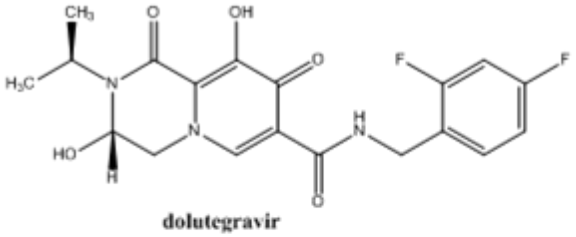
Darunavir is an oral second generation HIV protease inhibitor used in antiretroviral therapy (ART). It is generally quite tolerated from patients and exhibits fewer side effects than older antivirals of the same class, like indinavir. It is very usefull in the treatment of HIV-resistant patients.[65]



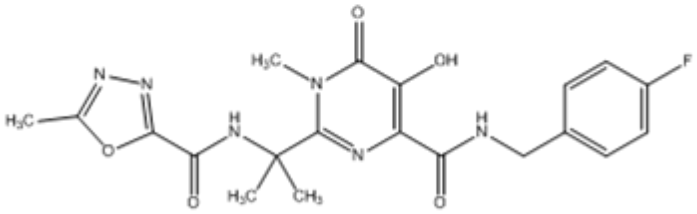
darunavir

4.2.4. HIV integrase inhibitors

Dolutegravir is a HIV-integrase inhibitor, a new class of anti-HIV drugs. Like all drugs of this category, it works by inhibiting viral enzyme integrase, which is responsible for the integration of the viral RNA into the chromosomes of the infected cells. In randomized control trials, it exhibited higher efficacy than raltegravir and efavirenz and did not showed any serious side effects. Dolutegravir could be administered once daily, without any pharmacological booster like ritonavir and also can be combined with other antiviral agents, *e.g.* abacavir and lamivudine in order to increase efficacy against HIV.
Raltegravir is the first integrase inhibitor approved. It was recently received approval for children aged 2-18 and it is available in chewable form. Unlike dolutegravir, it is administered twice a day and we are able to use it as monotherapy or in combination with other antiviral agents against HIV.[66]



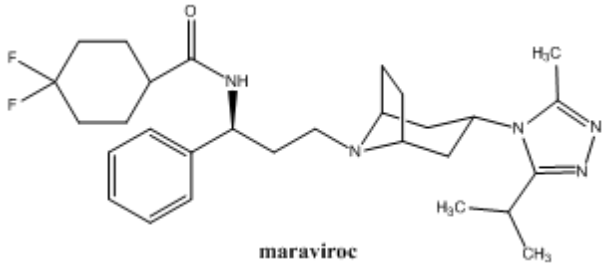
dolutegravir



raltegravir

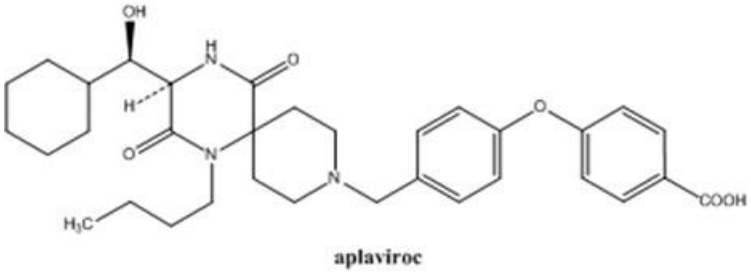
4.2.5. C-chemokine receptor type 5 antagonists

Maraviroc is an CCR5 (C-chemokine receptor type 5) antagonist and the only agent of this class that has been approved for the treatment of HIV infection and AIDS in adult patients with exclusively CCR5-tropic infection. CCR5 is a protein found on the white blood cells, which HIV virus uses in order to enter and infect the immune system cells. In placebo controlled studies, maraviroc seemed to be very effective and simultaneously safe enough with few side effects, but can lead to virologic failure and rebound HIV-1 viraemia.[67]

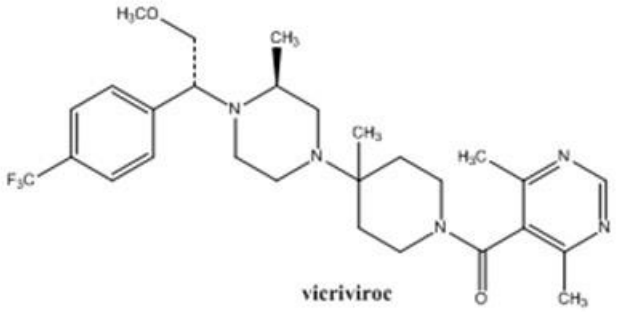


maraviroc

Aplaviroc and **vicriviroc** belong to CCR5 antagonists and are under clinical trials. Aplaviroc has shown severe hepatotoxicity, but vicriviroc was tolerated well combined with the pharmacological booster ritonavir.[68]



aplaviroc



vicriviroc

4.2.6. Other drugs

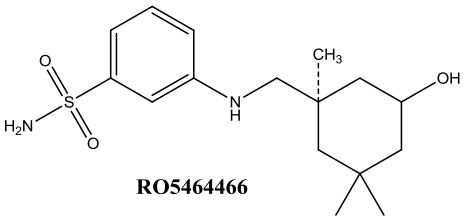
Scorpion venom peptide derivative Kn2-7 was designed based on BmKn2 peptide, in order to increase lipophilicity. After many trials and screening assays it was found that this peptide selectively inhibits HIV-1 and interacts in a direct way with the virus. It was also able to inhibit CXCR4-tropic replication of HIV-1 virus and with further investigation could be formed as a major antiviral agent against HIV-1.[69]

4.3. New drugs for influenza infections

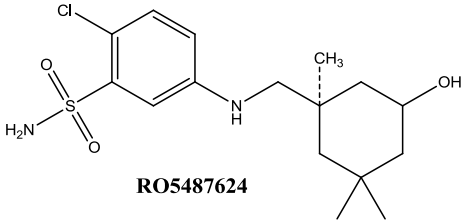
4.3.1. Benzenesulfonamide Derivatives Targeting Viral Hemagglutinin

Hemagglutinin (HA) is a glycoprotein present in the surface of influenza A virus. It "recognizes" sialic acid on the cell membranes and allows the fusion of the viral membrane with the host cell membrane. There are several types of hemagglutinin, but only H1, H2 and H3 are present in human influenza viruses.

As far as it concerns antiviral activity of benzenesulfonamide derivatives, recent pharmacokinetic studies shown that substances RO5464466 and RO5487624 lead to successful inhibition of Influenza A virus. Their efficacy was also tested *in vivo* and both were very active against H1N1 strain. RO5487624 possesses better pharmacokinetic properties and also better *in vitro* activity against Influenza virus.[70]



RO5464466

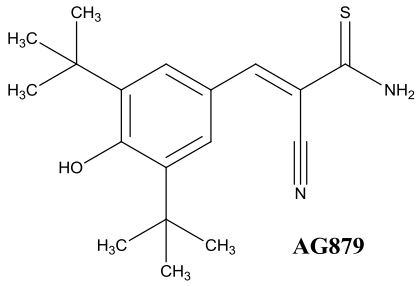


RO5487624

4.3.2. Receptor Tyrosine Kinase Inhibitors

Tyrosine kinase is an enzyme that involves in the phosphorylation of many proteins and takes part in the activation of signal transduction cascades. Drugs that use this mechanism usually are used as potent anticancer agents. However, recent studies assure that some newly developed compound could possess antiviral activities.

Substance AG879 – (*E*)-2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)prop-2-enethioamide – a tyrphostin-class RTKI, is under clinical trials and has shown inhibitory activity in the replication of many viruses including Influenza A and Herpes simplex. It's activity is tested both *in vivo* and *in vitro* and it showed low resistance (unlike amantadine), high selectivity and large spectrum of activity(against influenza A and B).[71]

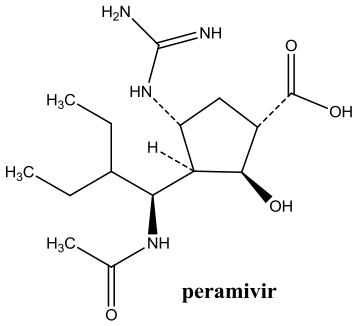


AG879

4.3.3. Neuraminidase inhibitors

Peramivir is a neuraminidase inhibitor that it is under clinical trials but has already been used in intravenous form for the treatment of H1N1 infection when the patients were unsuccessfully treated with other drugs, like oseltamivir. In recent clinical trials, patients who treated with peramivir present higher probability of been infected with

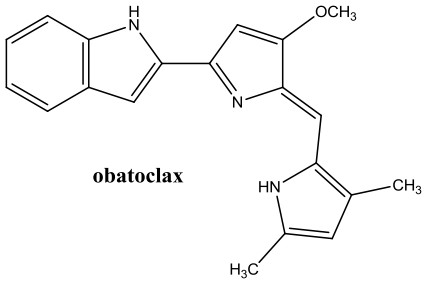
pneumonia. When the trials will be completed, it will be shown if peramivir deserves to be used in clinical practice.[72]



peramivir

4.3.4. Other drugs

Obatoclax is an experimental, candidate-drug for some cancer types like leukemia and lymphomas. However, it is approved that could also serve as an inhibitor of influenza A virus. Its mechanism of action includes binding to Mcl-1(myeloid cell leukemia sequence 1). The clinical trial was composed of chemical screening in human cells and revealed that obatoclax prevents the entry of Influenza virus into the cell by affecting transcription and translation of the viral RNA and consequently, by inhibiting viral protein synthesis.[73]

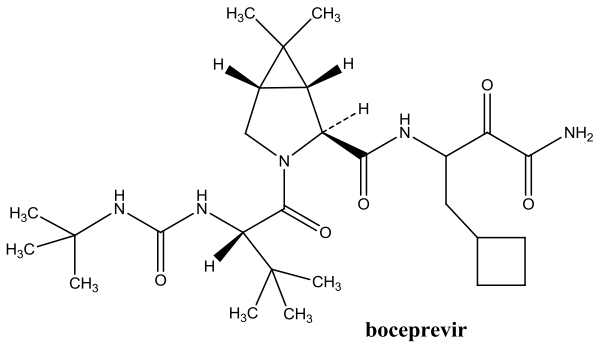


obatoclax

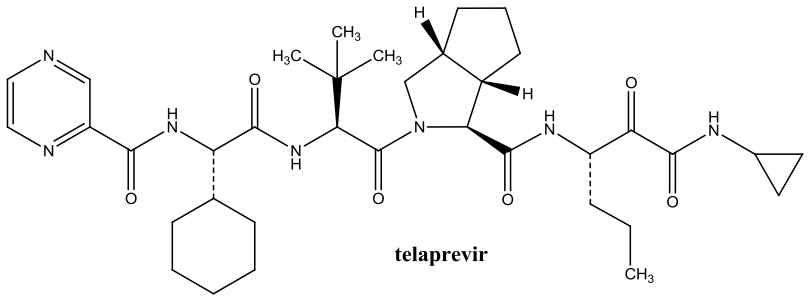
4.4. New drugs for Hepatitis C

4.4.1. Hepatitis C virus proteases inhibitors

This class of agents is also known as direct-acting antiviral drugs (DAA). Until now, there are two drugs that have been approved, **boceprevir** and **telaprevir**. They both work by inhibition of NS3/4A protease, which is necessary for viral replication and also are combined with pegylated interferon 2α and ribavirin. In randomized controlled trials, the drugs proved to be very effective against HCV and quite safe without serious side effects.[74]



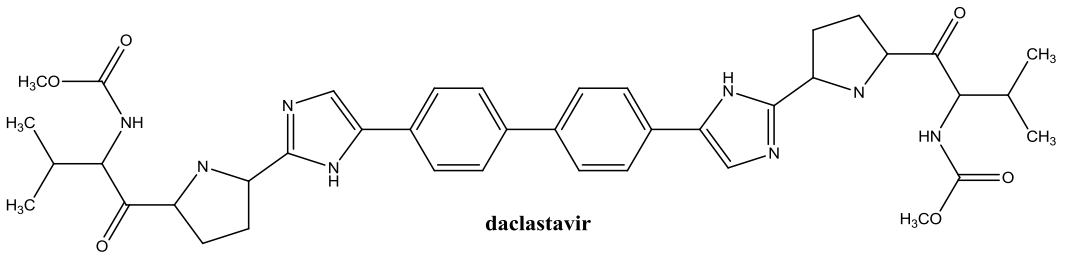
boceprevir



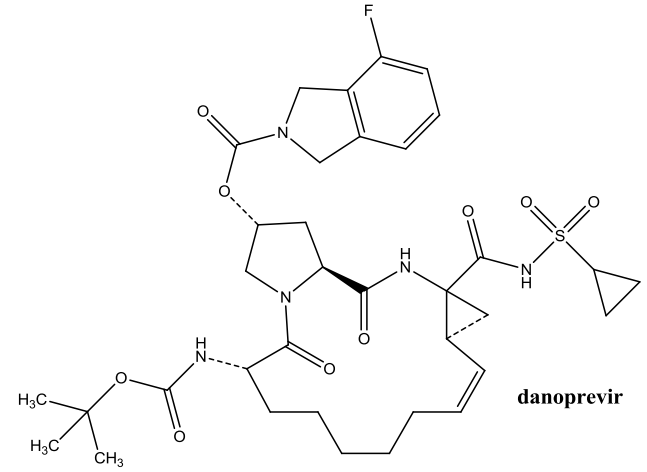
telaprevir

Other drugs of this class are under development.

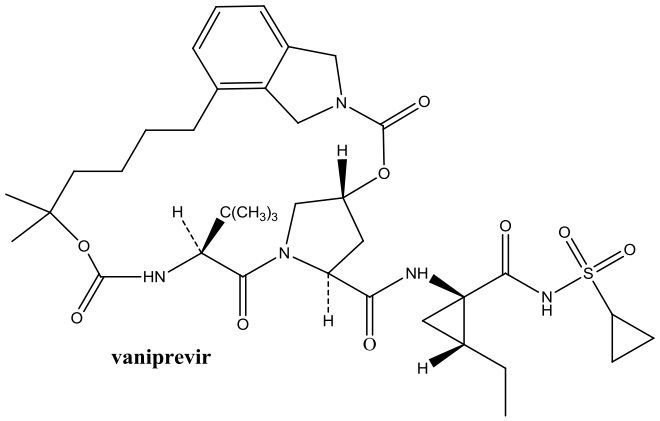
Daclastavir is the most potent NS5A inhibitor and showed a very good therapeutic index *in vitro.* It is very effectively distributed into the liver and it is available in oral dosage form. Similarly, **danoprevir**, **vaniprevir** and **asunaprevir** nowadays undergo clinical trials, in which it was found that they have a good therapeutic index and successfully inhibit replication of HCV virus. Asunaprevir, is an serine protease (NS3) inhibitor and its combinations with ribavirin and pegylated interferon 2α are tested.[75]



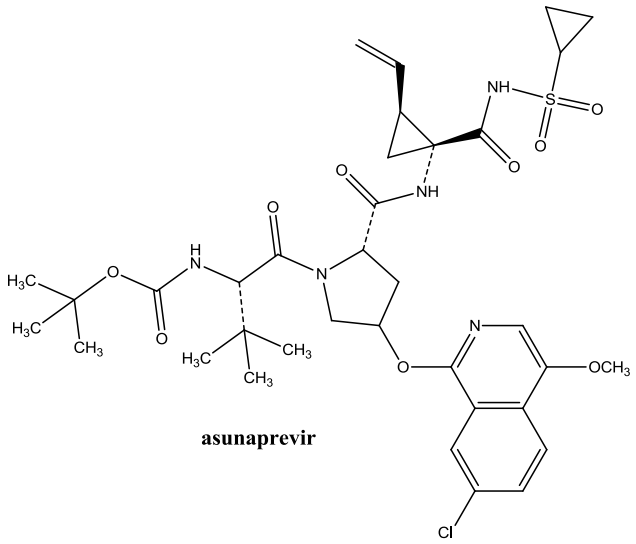
daclastavir



danoprevir



vaniprevir



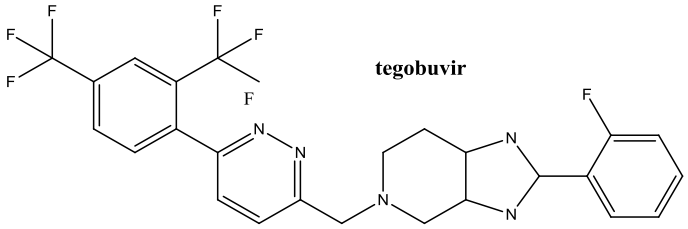
asunaprevir

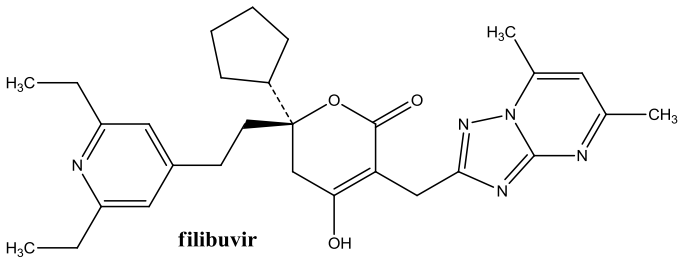
4.4.2. NS5B polymerase inhibitors

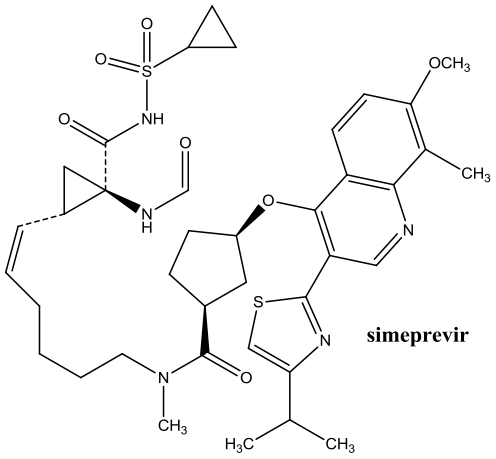
NS5B is an RNA polymerase and its primary role is in the replication of the viral genome. Inhibition of this enzyme is the target of new drugs concerning the treatment of chronic hepatitis C. There are two types of drugs in this class:

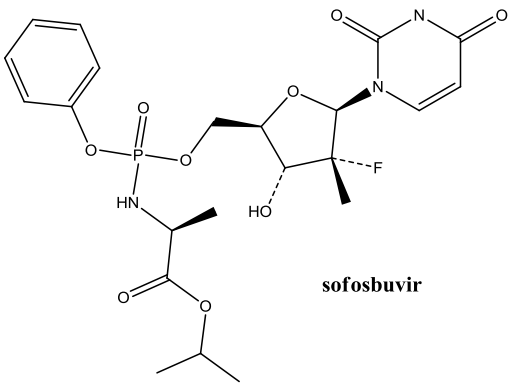
- Non-nucleoside type: **tegobuvir, filibuvir, simeprevir, sofosbuvir, acunaprevir**
- Nucleoside type: **mericitabine** [74]

The antiviral activity of **tegobuvir** has been tested in combination with ribavirin and pegylated interferon 2α. Results were positive and there was a significant increase in antiviral activity in the treated patients. **Filibuvir** underwent clinical trials, however recently was announced the termination of its development by the pharmaceutical company. Similarly for **simeprevir** and **sofosbuvir.** Recent data presented that a triple combination of sofosbuvir, ribavirin and **ledipasvir** lead to 100% treatment of HCV patients. [74,76]

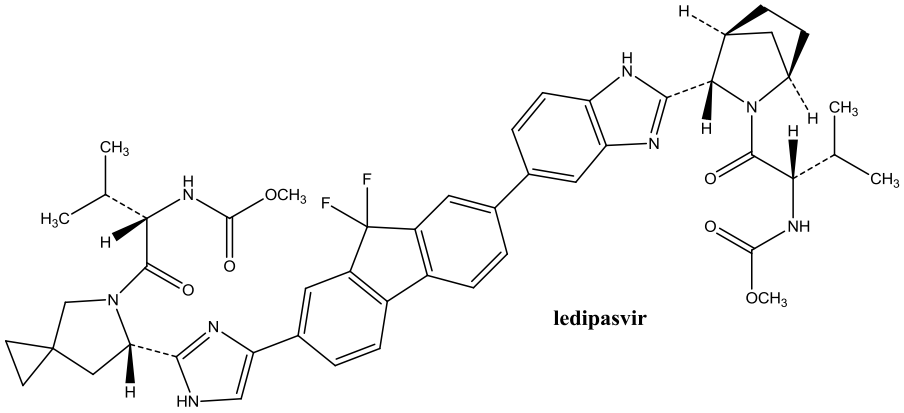






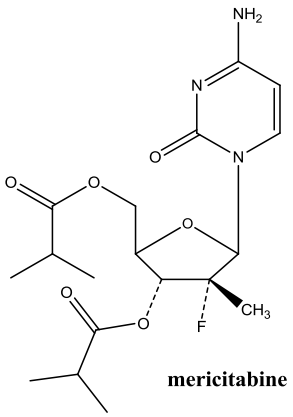


sofosbuvir



ledipasvir

Mericitabine is a nucleoside type of NS5B polymerase inhibitor that is in Phase II of clinical trials and has been tested similarly as previous agents in a combination with danoprevir and ritonavir (as a pharmaceutical booster).[74]

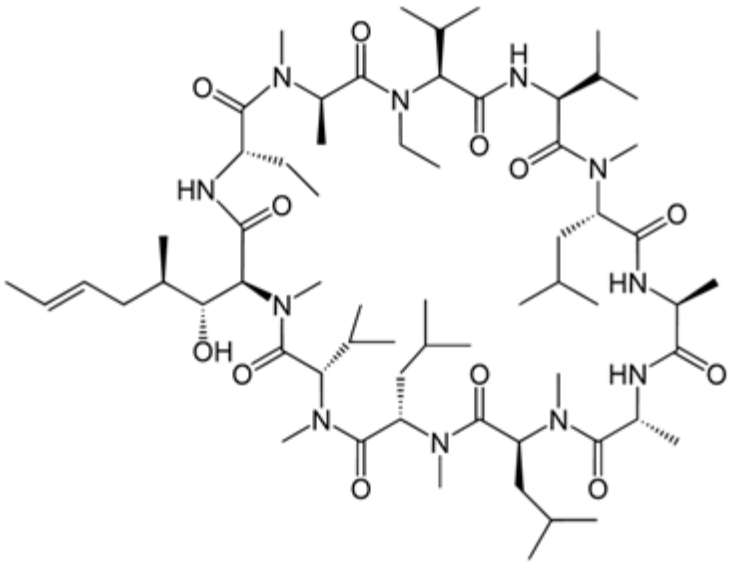


mericitabine

4.4.3. Cyclophilin inhibitors

Cyclophilin has a supporting role in the function of proteins and promotes the replication of HCV virus. In order to treat the disease, we need to inhibit the action of

cyclophilin. **Alisporivir** is a cyclophilin inhibitor, which can be administered orally and has been tested in HIV/HCV co-infected patients. Nowadays undergoes clinical trials and it is administered in a combination with ribavirin and pegylated interferon 2α with positive results against HCV virus.[74]



alisporivir

(Adopted from http://upload.wikimedia.org/wikipedia/commons/f/fa/Alisporivir.svg)

4.4.4. Other drugs

Miravirsen is the first microRNA-targeted drug that enters clinical trials. It has been designed to recognize the sequester miR-122, a liver specific microRNA, which HCV virus uses for its replication. In clinical trials, miravirsen has been given in subcutaneous injections and has shown a significant decrease in the amount of HCV RNA concentration in the serum. Fortunately, in the clinical trials there were not remarkably side effects. [74]

4.4.5. New combination therapies

In order to increase the therapeutic effect of the used treatment, sometimes a combination of three or more drugs is used. More frequent is the use of two DAA agents together with ribavirin and pegylated interferon 2α. It is found to be very effective in both genotypes (1 and 2) of the patients, which after an interval of 12 weeks they became HCV-RNA negative. Additionally, HCV protease inhibitors, such as boceprevir and telaprevir are combined with ribavirin and PEG interferon 2α, called

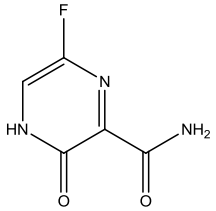
“triple therapy”. However, this type of treatment is difficult to manage group of patients with serious liver fibrosis or liver transplant failure. In clinical trials, is studied the possibility of the treatment of HCV without ribavirin. Data available from the trial, shown that combination of daclastavir with an under development NS3-4A inhibitor BMS-60032, has positive results in the patients infected with genotype 1 of HCV. [64]

As far as it concern new developed interferons, there is one attempt to enter the market pegylated interferon lambda 1. Unlike pegylated interferon 2α, the receptor of this new interferon is expressed in the hepatic cells and as a result, fewer side effects and flu symptoms are present during the treatment.[74]

4.5. New drugs for viral hemorrhagic fever

Ribavirin is the only registered drug for the treatment of viral hemorrhagic fever, however it shows serious adverse effects and it is not very effective in the treatment of severe cases.[77]

Favipiravir is an orally administered broad spectrum antiviral drug for the treatment of influenza. It is a pyrazine derivative (6-flouro-3-hydroxy-2-pyrazinecarboxamide) and *in vivo* experiments in guinea pigs has shown efficacy against flavivirus, arenavirus and phlebovirus. In two separated studies that were performed, favipiravir appeared to be highly effective against arenavirus infections and with further studies it will be a promising antiviral drug for the treatment of viral hemorrhagic fever.[78]



faripiravir

Furthermore studies have been made in order to find a way to prevent the entrance of Ebola virus into the cells. NPC1 (Niemann Pick C1) factor, which is located in lysosomes and it is critical for the entry of the virus. NPC1 interacts with GP (glycoprotein of Ebola virus), which is important for the fusion of the virus into the cells. These two molecules involve in the entry of the Ebola virus with a complex mechanism that need further studies to be determined. A new molecule, U18666A is already in the phase of development and could be used as an antiviral agent by blocking

of Ebola GP, although many more studies are required in order to be determined if it would be safe and effective enough.[79]

5. DISCUSSION

Since 1960's, where the first antiviral drug was developed, a great progress has been made and many useful drugs against many serious viral diseases have been developed. Although antiviral drugs do not kill or remove the virus from the body, they have significantly increased the life expectancy in many lethal diseases, such as hepatitis C and AIDS. Old drugs have, sometimes, presented low efficacy and undesirable side effects and that is the reason why newer and safer antivirals were developed, which also possess broader spectrum of activity.

Many substances undergo nowadays clinical trials in order to be determined whether they could be used as antiviral drugs. Many of them, such as favipiravir, dolutegravir, peramivir have shown great results both in animal testing and in patients. On the other hand, some others need further studies and experiments before authorization and marketing.

There are many viral diseases, which could be only prevented by vaccines and not treated with certain drugs. The case of HPV (Human Pappilomavirus) is the most significant. The virus infects the body and stays there during the lifetime and the person presents warts in different parts of its body, *e.g.* genital and common warts. This type of infection is the number one cause for cervical cancer, but could be prevented by vaccines Gardasil [80] and Cervarix.[81]

Generally, many vaccines have been developed for many viral diseases, like influenza, yellow fever, hepatitis B and C, which gave the opportunity to protect vulnerable groups of people from been infected by several viruses. [82-84] Unfortunately, for diseases such as AIDS, there are not any vaccines registered and protection could be achieved by the usage of condoms.

6. CONCLUSION

The aim of my work was to present new antiviral drugs that have been developed since 2010 till 2013 or are under clinical trials and also to mention older antivirals.

New developed drugs have greater efficacy and less side effects that older ones. Thanks to them, the life expectancy has been significantly increased and the quality of life has been improved.

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